Original article

Evaluation of Angiotensin I-Converting Enzyme (ACE) inhibitory potential of some underutilized indigenous fruits of West Bengal using an *in vitro* model

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Evaluation of Angiotensin I-Converting Enzyme (ACE) inhibitory potential of some underutilized indigenous fruits of West Bengal using an *in vitro* model.

Abstract – Introduction. Angiotensin I-Converting Enzyme (ACE) is a key component in regulation of blood pressure by virtue of the rennin-angiotensin system. ACE converts the inactive decapeptide, angiotensin I, into the potent vasopressor octapeptide, angiotensin II, and inactivates bradykinin, which has a vasodilating action. So, inhibition of ACE has become a major target in control of hypertension. It is well known that the consumption of fruits could provide health benefits by lowering the risk of chronic diseases such as metabolic syndrome diseases including type 2 diabetes and cardiovascular disease. Our current study was focused on investigating the ACE inhibitory property of a few underutilized minor fruits of West Bengal using an *in vitro* assay. **Materials and methods**. The potential antihypertensive activity of underutilized indigenous edible fruits of West Bengal, India, was evaluated by their ability to inhibit Angiotensin-Converting Enzyme (ACE). The ACE inhibitory property was assayed using ACE from rabbit lung and hippuryl-histidyl-leucine as the substrate. Nineteen fruit species belonging to 15 families were investigated. The percentage ACE inhibitory activities of these fruits were studied at 20 µg fresh weight of fruit extract per mL. The total phenol content of all these fruits was determined following the standard Folin-Ciocalteu method. Total flavonoid content was also measured. **Results**. The aqueous fruit extracts of the red variety of *Trapa bispinosa*, *Phoenix sylvestris*, *Cicca acida*, *Achras sapota* and *Averrhoa carambola* presented more than 75% ACE inhibition. On the other hand, *Punica granatum*, *Spondias pinnata*, *Trapa bispinosa* (green) and *Ziziphus mauritiana* showed about 50% inhibition. *Aegle marmelos*, *Annona squamosa, Annona reticulata, Feronia elephantum, Physalis peruviana* and *Syzygium jambos* showed low activity (< 50% inhibition). **Discussions**. To date there has been no report on Angiotensin I-Converting Enzyme inhibitory activities of these underutilized minor fruits of West Bengal, India. During our study no correlation could be established between the % ACE inhibition and the total phenol or flavonoid content of these fruit extracts. So, it appears that non-phenolic components may also be responsible for ACE inhibitory activity. In our investigation we tried to establish the fact that the consumption of these underutilized minor fruits might have potential in managing cardiovascular diseases.

India / West Bengal / natural resources / fruit trees / fruits / enzyme activity

Évaluation du potentiel inhibiteur de l'enzyme de conversion de l'angiotensine I (ECA) dans certains fruits indigènes sous-utilisés du Bengale occidental, à l'aide d'un modèle *in vitro*.

Résumé – Introduction. L'enzyme de conversion angiotensine I (ECA) est un élément clé dans la régulation de la pression artérielle, en vertu du système rénine-angiotensine. L'ECA convertit le décapeptide inactif, l'angiotensine I, en un puissant octapeptide vasoconstricteur, l'angiotensine II, et inactive la bradykinine qui a une action vasodilatatrice. Ainsi, l'inhibition par l'ECA est devenue un important objectif de contrôle de l'hypertension. Il est bien connu que la consommation de fruits pourrait avoir un effet bénéfique sur la santé en réduisant le risque de maladies chroniques liées au syndrome métabolique dont les diabètes de type 2 et les maladies cardiovasculaires. Notre étude a été axée sur l'évaluation de la propriété d'inhibition de l'ECA de quelques fruits mineurs sous-utilisés du Bengale occidental en utilisant un dosage *in vitro*. **Matériel et méthodes**. L'activité antihypertensive potentielle de fruits comestibles sous-utilisés indigènes du Bengale occidental (Inde) a été évaluée par leur capacité à inhiber l'enzyme de conversion de l'angiotensine (ECA). La propriété inhibitrice de l'ACE a été analysée en utilisant l'ACE extrait de poumon de lapin et l'hippuryl-histydyl-leucine comme substrat. Dix-neuf espèces fruitières appartenant à 15 familles ont été étudiées. Le taux d'activités d'inhibitrices de l'ECA de ces fruits a été étudié avec 20 mg d'extrait de fruit (poids frais) par mL. La teneur totale en phénol de tous ces fruits a été déterminée en suivant la méthode standard de Folin-Ciocalteau. La teneur totale en flavonoïdes a également été mesurée. **Résultats**. Les extraits aqueux de fruits de *Trapa bispinosa* (variété rouge), ainsi que ceux de *Phoenix sylvestris*, *Cicca acida*, *Achras sapota* et *Averrhoa carambola* ont présenté une activité inhibitrice de l'ECA de plus de 75 %. Par ailleurs, *Punica granatum*, *Spondias pinnata*, *Trapa bispinosa* (variété verte) et *Ziziphus mauritiana* ont montré une activité inhibitrice d'environ 50 %, alors que les espèces *Aegle marmelos*, *Annona squamosa, Annona reticulata, Feronia elephantum, Physalis peruviana* et *Syzygium jambos* ont montré une faible activité inhibitrice (< 50 %). **Discussions**. Jusqu'à présent, il n'existait pas de publications sur l'activité inhibitrice de l'enzyme de conversion angiotensine I de ces fruits mineurs sous-utilisés du Bengale occidental. Lors de notre étude, aucune corrélation n'a pu être établie entre le taux d'inhibition de l'ECA et les teneurs en flavonoïdes ou phénols totaux de ces extraits de fruits. Il semble donc que des composants non-phénoliques pourraient également être responsables de l'activité inhibitrice de l'ECA. Nos recherches conduiraient à montrer que la consommation de ces fruits mineurs sous-utilisés pourrait potentiellement intervenir dans la gestion des maladies cardio-vasculaires.

Inde / Bengale occidental / ressource naturelle / arbre fruitier / fruits / activité enzymatique

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1. Introduction

Angiotensin I-Converting Enzyme (ACE; kinase II; EC3.4.15.1) is potentially of great importance for controlling blood pressure by virtue of the rennin-angiotensin system [1–3]. ACE converts the inactive decapeptide, angiotensin I, into the potent vasopressor octapeptide, angiotensin II, and inactivates bradykinin, which has a vasodilating action [4]. It is well known that the consumption of fruits could provide health benefits by lowering the risk of chronic diseases such as metabolic syndrome diseases including type 2 diabetes and cardiovascular disease [5–7]. One of the long-term complications of type 2 diabetes is high blood pressure, or hypertension [8]. Hypertension has become the most common serious chronic health problem globally in recent years. According to the World Health Organization (WHO), around 20–45% of a population and nearly 50–60% of elderly people have elevated blood pressure [9]. Angiotensin I-converting enzyme (ACE) is an important enzyme involved in maintaining vascular tension by two different reactions. This enzyme catalyzes: (1) conversion of the inactive angiotensin I into a powerful vasoconstrictor and promoter of sodium retention, angiotensin II, and (2) inactivation of the vasodilator bradykinin, which is conducive to lower blood pressure [10]. Inhibition of ACE is considered to be a useful therapy in the control of blood pressure in hypertensive patients. Therefore, dietary sources of ACE inhibitors are potentially beneficial [10].

ACE inhibitors have proven to be very effective in the treatment of heart failure caused by systolic dysfunction (*e.g.*, dilated cardiomyopathy) [11]. Specific drugs used in blocking the formation of angiotensin II include captopril, benazapril, enalapril, fosinopril, lisinopril, ramipril, etc. However, these synthetic drugs are believed to have certain side effects such as hypotension, reduced renal function, dry cough, skin rashes, taste disturbances and fetal abnormalities [11]. Therefore, for safe and economical use, interest in identifying food sources as ACE inhibitors has increased. ACE inhibitors from food sources include a peptide from corn gluten digested by pescalase [12], a peptide isolated from an acid extract of tuna [13], and various flavonoids isolated from leaves of persimmon [14, 15]. However, there has been no study on the ACE inhibitory activity of underutilized indigenous fruits of West Bengal. A number of compounds from different plants have been identified to possess *in vitro* ACE inhibitory activity. These include hydrolyzable tannins, phenylpropanes, proanthocyanidins, flavonoids, xanthones, fatty acids, terpenoids, alkaloids, oligosaccharides and peptide amino acids [16–18].

2. Materials and methods

2.1. Fruit materials

Nineteen minor fruits, indigenous to West Bengal and belonging to 15 families, were selected for investigation. These investigated fruits were *Achras sapota* L. (Sapotaceae), *Aegle marmelos* (L.) Corr. Serr. (Rutaceae), *Annona reticulata* L., *Annona squamosa* L. (Annonaceae), *Averrhoa carambola* L. (Oxalidaceae), *Borassus flabellifer* L. (Arecaceae), *Carissa carandas* L. (Apocynaceae), *Cicca acida* (L.) Merr (Euphorbiaceae), *Citrus decumana* (L.) Murr. (Rutaceae), *Feronia elephantum* Correa. (Rutaceae), *Grewia asiatica* L. (Malvaceae), *Nephelium longana* (Lam.) Cam (Sapindaceae), *Phoenix sylvestris* Roxb. (Arecaceae), *Physalis peruviana* L. (Solanaceae), *Punica granatum* L. (Lythraceae), *Spondias pinnata* (L.f) Kurz. (Anacardiaceae), *Syzygium jambos* L. (Alston) (Myrtaceae), red and green varieties of *Trapa bispinosa* Roxb. (Trapaceae), and *Ziziphus mauritiana* Lam. (Rhamnaceae). All these fruits were collected just at their ripe stage (*i.e.*, ready for consumption) from local markets of North Kolkata, West Bengal, India, as reported in Das *et.al.* [19].

2.2. Preparation of fruit extract

The juicy pulpy or dry scaly edible portion of each fruit was weighed, crushed and

warmed at 100 °C with double-distilled water for 5–10 min to inactivate enzymes present in the fruit materials and to extract the active constituents. Then it was strained through a sieve and the extract obtained was centrifuged at 10,000 rpm for 15 min. These final supernatants were preserved at –20 °C to study their enzyme inhibitory activities. The dilution of the aqueous extract was prepared with double-distilled water. The concentration of each extract was expressed in terms of weight of fresh fruit to make extract per mL. The concentration used during the assay was 20 µg·mL^{-1}.

2.3. Chemicals

Angiotensin-converting enzyme from rabbit lung and hippuryl-histidyl-leucine (HHL) were obtained from Sigma, USA. The standard ACE inhibitor drug used was lisinopril obtained from Lupin Pharmaceuticals (India). All the other reagents were of analytical grade.

2.4. ACE inhibition assay

The Angiotensin-Converting Enzyme (ACE) inhibitory property was assayed by the modified method of Cushman and Cheung [20] using hippuryl-histidyl-leucine (HHL) as a substrate. Angiotensin-Converting Enzyme (ACE) from rabbit lung was prepared in 200 mM borate buffer (pH 8.3) at a concentration of 100 mU·mL^{-1}. A reaction mixture containing 0.25 mL 7 mM HHL in pH 8.3 borate buffer (200 mM), 0.2 mL 2 M NaCl, 0.02 mL H_2O, 0.015 mL aqueous fruit extract and 0.015 mL 100 mU·mL^{-1} of ACE (in the pH 8.3 buffer) was incubated at 37 °C for 30 min. The reaction was stopped by adding 0.25 mL 1(N) HCl. The hippuric acid liberated from the HHL by ACE was extracted with ethyl acetate (1.5 mL). An aliquot of the extract (1.3 mL) was evaporated to dryness and the residue was dissolved in 0.4 mL H_2O. The control set contained 0.015 mL of distilled water instead of fruit extract. The hippuric acid concentration was determined by measuring the absorbance spectrophotometrically at 228 nm against a blank solution similarly prepared by adding buffer instead of ACE. Assays were carried out at least in triplicate. The percentage inhibition of ACE activity by plant extracts was calculated by the formula $[(A_0 - A_e) / A_0] \times 100$ (A_0 = absorbance without extract; A_e = absorbance with extract). The % inhibitions of different fruit extracts were compared with the % inhibition value of the standard cardioprotective drug lisinopril.

2.5. Determination of total phenol content

Total phenol content was determined by Folin-Ciocalteu reagent in alkaline medium [21] and was expressed as gallic acid equivalents (GAE) (equivalent to µg gallic acid per mg fresh fruit weight). Total phenol content was calculated from the regression equation (y = 0.0193 x – 0.0006) prepared from a range of concentrations of gallic acid and optical densities for the concentrations.

2.6. Determination of total flavonoid content

Total flavonoid content was determined following Kim *et al.* [22] and was expressed as catechin equivalent (CE) (equivalent to µg catechin per mg fresh fruit weight), calculated from the regression equation (y = 0.024 x – 0.0089) prepared from a range of concentrations of catechin and optical densities for the concentrations.

2.7. Statistical analysis

Results are expressed as mean ± standard deviation. The data was statistically analyzed using Student's t-test (MS Excel 2007: p values < 0.05 considered significant).

3. Results

Of the 19 different species of indigenous fruits of West Bengal studied, fifteen species showed inhibition against Angiotensin-Converting Enzyme (ACE) at a concentration of 20 µg fresh fruit·mL^{-1} aqueous extract; the other five species showed no inhibition against this enzyme (*figure 1*).

Among the 15 species which showed an inhibitory property at 20 µg fresh fruit·mL^{-1}

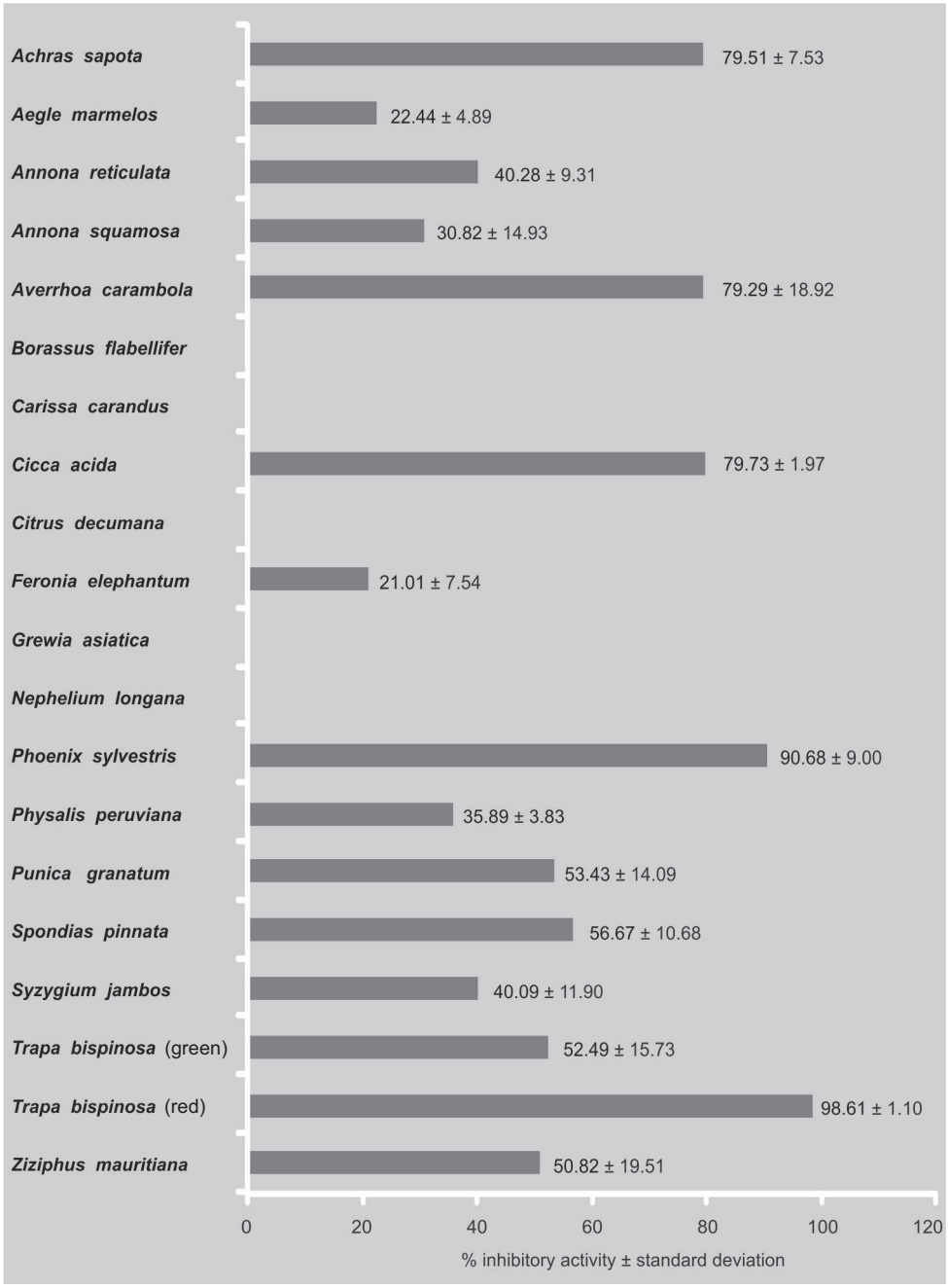

Figure 1.
Angiotensin I-Converting Enzyme (ACE) inhibitory activity (%) of 19 underutilized indigenous fruits of West Bengal (India) assayed *in vitro*.

aqueous extract, the red variety of *Trapa bispinosa* showed the maximum inhibition [about (98.61 ± 1.1)% inhibition]; it was followed by *Phoenix sylvestris* [(90.68 ± 9)% inhibition]. *Achras sapota*, *Averrhoa carambola* and *Cicca acida* also showed high inhibitory activity [(79.51 ± 7.53)%, (79.29 ± 18.92)% and (79.73 ± 1.97)%, respectively]. On the other hand, the four species *Punica granatum*, *Spondias pinnata*, *Trapa bispinosa* (green) and *Ziziphus mauritiana* showed about 50% inhibition against ACE [(53.43 ± 14.09)%, (56.67 ± 10.68)%, (52.49 ± 15.73)% and (50.82 ± 19.50)%, respectively]. *Aegle marmelos*, *Annona squamosa, Annona reticulata, Feronia elephantum,*

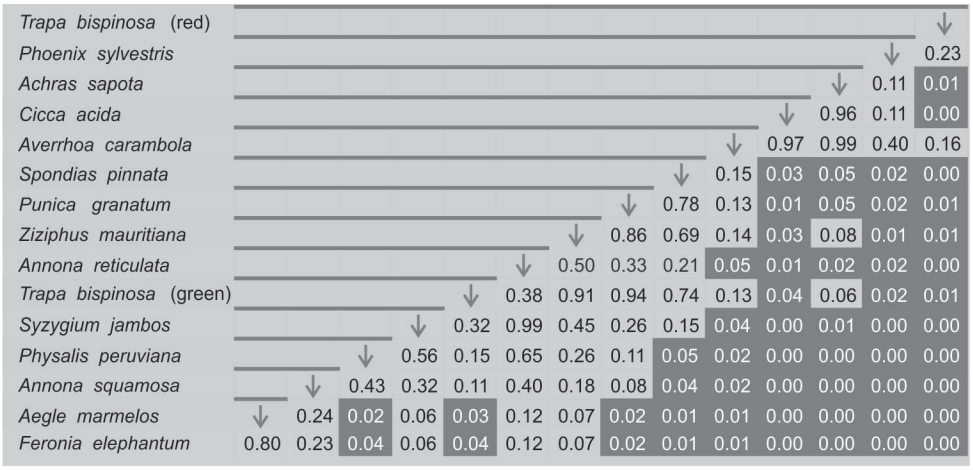

Figure 2.
Differences in Angiotensin I-Converting Enzyme (ACE) inhibitory activity among 15 underutilized indigenous fruits of West Bengal (India) assayed *in vitro* and measured by the *t*-test. Significant differences are highlighted in gray (p-values < 0.05).

Physalis peruviana and *Syzygium jambos* showed low inhibitory activity. *Borassus flabellifer, Carissa carandus, Citrus decumana, Grewia asiatica* and *Nephelium longana* had no activity at the concentration tested. ACE inhibitory activity among the fruits studied by the *t*-test and the *p*-values showed significant differences (*figure 2*). The % ACE inhibition by lisinopril at 13 $\mu g \cdot mL^{-1}$ concentration was found to be (99.29 ± 1.11)%.

Different flavonoids, as well as plant extracts rich in flavonoids, were found to be effective ACE inhibitors both *in vitro* and *in vivo* [23]. During our present study, the total phenol and flavonoid contents of the 19 fruits studied [19] showed no correlation between ACE inhibitory activity and total phenol (R^2 = 0.0034) and flavonoid contents (R^2 = 0.134).

4. Discussion

Consumption of fruits and vegetables could be an effective way to prevent nutritional deficiencies and chronic diseases [24].

The potential cardiovascular protective properties of aqueous and 70% ethanol extracts from *Actinidia deliciosa* Lindl. (Actinidiaceae), well known as "kiwi", a plant native to Eastern Asia, have been reported. An inhibitory activity against angiogensin I-converting enzyme of kiwi fruit extracts has been reported [25].

The aqueous extract from *Berberis vulgaris* L. (Berberidaceae) fruit was tested to evaluate its antihypertensive effects on DOCA-induced hypertension in rats, and the results suggest that the antihypertensive and vasodilatory effects of *B. vulgaris* fruit extract are mainly endothelial-independent and it may be used to treat hypertension, a status with endothelial dysfunction [26].

Elettaria cardamomum (L.) Maton. (Zingiberaceae), small cardamom, fruit powder is reported for its antihypertensive potential and its effect on some of the cardiovascular risk factors in individuals with stage 1 hypertension [27]. The antihypertensive effect of pink guava (*Psidium guajava* Mill.) (Myrtaceae) puree on Spontaneous Hypertensive Rats (SHR) has also been reported [28]. In limited studies in human and murine models, *Punica granatum* L. (Lythraceae) or pomegranate juice has been shown to exert significant antihypertensive effects [29]. The efficacy of Indian gooseberry (*Phyllanthus emblica* L.) (Phyllanthaceae), guava (*Psidium guajava*), wood apple (*Limonia acidissima* L.) (Rutaceae) and star fruit (*Averrhoa carambola* L.) (Oxalidaceae) for angiotensin-converting enzyme (ACE) inhibitory activity using hippuryl-L-histidyl-L-leucine as a substrate was determined [30].

Fruits of seven fully ripened strawberry (*Fragaria* × *ananassa* Duch.) (Rosaceae) cultivars grown in Brazil (Dover, Camp Dover, Camarosa, Sweet Charlie, Toyonoka, Oso Grande and Piedade) were evaluated for ACE relevant for potentially managing

hypertension. The evaluated cultivars had no significant ACE inhibitory activity, reflecting low antihypertensive potential [31].

An apple (*Malus domestica* Borkh.) (Rosaceae) skin extract rich in flavonoids, the major constituents of the extract and their selected metabolites were assessed for the ACE inhibitory property *in vitro* [23]. It has been reported that ACE inhibitory activity of the fruit could be due to the varied amounts of phytoconstituents present in the extracts, *i.e.*, phenols, flavonoids, ascorbic acid and protein contents [23].

To date there has been no report on Angiotensin–I Converting Enzyme inhibitory activities of the underutilized minor fruits of West Bengal, India. Making an inventory of the underutilized edible fruits would help in conservation and valorization measures [32, 33].

During our present study, no correlation could be established between the % ACE inhibition and the total phenol and/or flavonoid content of 19 underutilized fruit extracts. However, the ACE inhibition activities of some of these fruit extracts were found to be very good. Extracts from 20 µg fruit of *Trapa bispinosa* (red) was equivalent to the activity of 13 µg lisinopril. A much higher amount of the fruit is consumed in a day. Each fruit weighs 4–5 g. We found that activity of *Phoenix sylvestris* was not significantly different from that of *T. bispinosa*. So, from our results, it appears that non-phenolic components can also be responsible for ACE inhibitory activity. In our investigation, we tried to establish the fact that consumption of underutilized minor fruits from West-Bengal might have potential in managing cardiovascular diseases. So, further *in vivo* studies are required to find out their efficacy, and identification of the active constituents responsible for such inhibition are required to be analyzed.

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Evaluación del potencial inhibidor de la enzima convertidora de la angiotensina I (ECA) en determinadas frutas locales infrautilizadas de Bengala occidental, mediante un modelo *in vitro*.

Resumen – **Introducción**. La enzima convertidora de la angiotensina I (ECA) es un elemento clave en la regulación de la presión arterial, en virtud del sistema renina angiotensina. La ECA convierte el decapéptido inactivo, la angiotensina I, en un potente octapéptido vasoconstrictor, la angiotensina II, e inactiva la bradiquinina, que tiene una acción vasodilatadora. Así, la inhibición de la ECA se ha convertido en un importante objetivo para controlar la hipertensión. Se sabe que el consumo de frutas podría tener un efecto beneficioso para la salud, reduciendo el riesgo de enfermedades crónicas ligadas al síndrome metabólico, entre ellas la diabetes de tipo 2 y las enfermedades cardiovasculares. Nuestro estudió se basó en la evaluación de las propiedades inhibidoras de la ECA de algunas frutas menores infrautilizadas en Bengala occidental mediante una dosificación *in vitro*. **Material y métodos**. La actividad antihipertensiva potencial de las frutas comestibles infrautilizadas de Bengala occidental (India) se evaluó por su capacidad inhibidora de la enzima convertidora de la angiotensina (ECA). Se analizó la propiedad inhibidora de la ACE con ACE extraída de pulmón de conejo e hipuril-histidil-leucina como sustrato. Se estudiaron 19 especies de frutas pertenecientes a quince familias. El índice de actividades inhibidoras de la ECA de dichas frutas se estudió con 20 mg de extracto de fruta (peso fresco) por ml. El contenido total en fenoles de dichas frutas se determinó con el método estándar de Folin-Ciocalteau. También se midió el contenido total en flavonoides. **Resultados**. Los extractos acuosos de frutas de *Trapa bispinosa* (variedad roja), así como los de *Phoenix sylvestris*, *Cicca acida*, *Achras sapota* y *Averrhoa carambola* presentaron una actividad inhibidora de la ECA de más del 75%. Asimismo, *Punica granatum*, *Spondias pinnata*, *Trapa bispinosa* (variedad verde) y *Ziziphus mauritiana* mostraron una actividad inhibidora de alrededor del 50%, mientras que las especies *Aegle marmelos*, *Annona squamosa, Annona reticulata, Feronia elephantum, Physalis peruviana* y *Syzygium jambos* mostraron una débil actividad inhibidora (< 50%). **Discusión**. Hasta este momento no existían publicaciones sobre la actividad inhibidora de la enzima convertidora de la angiotensina I de estas frutas menores infrautilizadas de Bengala occidental. Tras nuestro estudio, no se ha podido establecer ninguna correlación entre el índice de inhibición de la ECA y el contenido en flavonoides o fenoles totales de dichos extractos de frutas. Por tanto, parece que los componentes no fenólicos podrían ser igualmente responsables de la actividad inhibidora de la ECA. Nuestras investigaciones pretenderían demostrar que el consumo de estas frutas menores infrautilizadas podría potencialmente intervenir en el tratamiento de enfermedades cardiovasculares.

India / Bengala Occidental / recursos naturales / árboles frutales / frutas / actividad enzimática